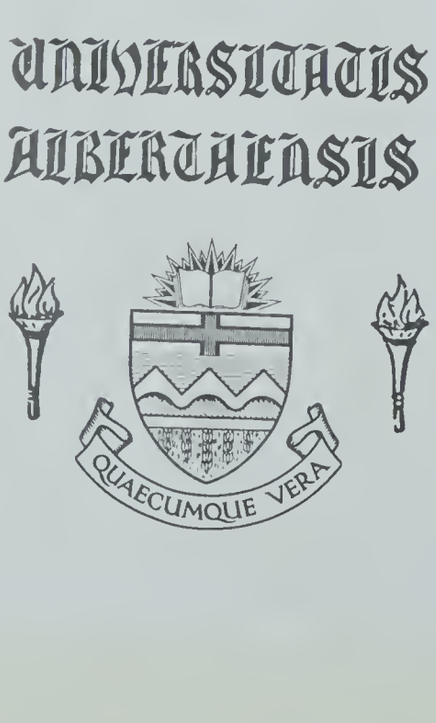

THE UNIVERSITY OF ALBERTA

STUDIES ON THE ROLE OF BIOTIN IN THE NUTRITION OF TURKEY POULTS

by

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "Studies on the Role of Biotin in the Nutrition of Turkey Poults" submitted by Samlal Jaikaran, B.Sc. (Hons), in partial fulfilment of the requirements for the degree of Master of Science.

ABSTRACT

Experiments were conducted to develop a purified ration for poults and with it to study the biotin requirements of turkey poults. The biotin content of 15 feed ingredients and 6 commercial feeds was determined using a microbiological assay. Studies were also conducted on the effect of progression of the hatching season on the biotin content of yolks of turkey eggs and storage of biotin in livers of day-old poults. The effect of dietary biotin level and ration type on storage of biotin in livers of 4-week-old poults was determined.

A purified ration designed to provide the nutrient requirements of the poult was formulated. When biotin was omitted from the ration severe symptoms of biotin deficiency occurred. When graded levels of biotin were added, incidence of deficiency symptoms was reduced as level of supplementation was increased but the amount of added biotin required to prevent deficiency symptoms varied between 100 and 300 ug of biotin per kg of ration in different feeding trials.

The biotin content of 15 feed ingredients and 6 commercial turkey starter rations was determined. The biotin content of the feed ingredients was similar to values reported in the literature. Levels of biotin found in commercial feeds varied considerably but all appeared to contain a sufficient amount to meet the requirement of the poult for biotin.

The biotin content of yolks of hatching (turkey) eggs and livers of day-old poults, taken at 28 day intervals during the hatching season, was determined. The biotin content of the yolks remained relatively constant throughout the hatching season but storage of biotin in livers of day-old poults declined steadily as the hatching season progressed.

The influence of protein supplements, addition of biotin to the ration and progression of the hatching season on performance of poults and storage of biotin in the liver was studied. When meat meal and fish meal were the only protein supplements included in a practical type ration, without added biotin, severe symptoms of a biotin deficiency occurred, rate of growth was depressed and biotin content of the liver was low. Addition of biotin to the ration greatly reduced symptoms of deficiency and increased biotin storage in the liver but failed to increase rate of growth. When soybean meal was included in the protein supplement, rate of growth was increased, symptoms of a biotin deficiency were absent and the biotin content of livers was increased. Addition of biotin to the ration had no effect on rate of growth but liver storage of biotin was increased. When graded levels of biotin were added to a purified ration, deficient in biotin, the amount of biotin found in the livers of poults at 4 weeks of age tended to increase as the biotin content of the ration was increased.

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INTRODUCTION

During the past twenty years, turkey producers in Alberta have experienced heavy losses from a disorder that occurs in turkey poults. The symptoms of the disorder, which appears when poults are from two to four weeks of age, include the following: lack of uniformity, broken feathers, hock disorder, dermatitis and diarrhoea. Rate of mortality in affected poults is usually higher than in non-affected poults and many poults are destroyed because of hock disorder or stunted growth.

Although considerable success in preventing the condition has been achieved by supplementing starter rations with biotin, the disorder in the field does not always appear to be a simple biotin deficiency. It has been generally noted that the incidence and severity of the disorder is greater late in the hatching season. It has also been observed that the occurrence of the condition is somewhat sporadic. Some producers suffer heavy losses, while others, using the same ration and the same strain of turkeys, have little or no difficulty.

A similar pattern has been encountered in experimental work. In some instances symptoms of the disorder were severe, in others, they were slight or even absent. In addition, the overall problem has been confounded by the fact that most of the symptoms noted are common to a number of other deficiencies. Therefore, the need for more definitive information relating to the disorder became important. Consequently, experiments were undertaken to obtain information on the role of biotin in turkey nutrition.

REVIEW OF LITERATURE

A. Historical

The discovery that biotin is a vitamin resulted from experiments designed to study the nature of the toxicity caused by feeding raw egg white to experimental animals and from growth and respiration studies with legume nodule microorganisms. Bateman (1916) observed that raw egg white when included in the diets of rats and dogs caused a definite toxicity. Later investigations of the nature of the toxicity led to the demonstration that an organic factor existed in natural feedstuffs such as liver and yeast which protected the rat against the toxicity caused by feeding raw egg white. The protective factor was called 'factor X' by Boas (1927), 'vitamin H' by György (1931 - cited by Ochoa and Kaziro, 1965) and the 'anti-egg-white injury factor' by Lease and Parsons (1934). Ringrose, Norris and Heuser (1931) reported the occurrence of a pellagra-like syndrome in chicks fed purified rations containing either air-dried egg albumin or purified casein. The symptoms were prevented by addition of autoclaved yeast or milk vitamin concentrate to the rations. Allison, Hoover and Burk (1933) and Allison and Hoover (1934) reported that a factor isolated from yeast was required for growth and respiration of many strains of Rhizobia bacteria. This factor was named 'coenzyme R.' Kögl and Tönnis (1936) isolated a pure crystalline fraction from egg yolk which strongly stimulated the growth of yeast. The active substance was named biotin.

Subsequent investigations led to the conclusion that biotin was identical with 'coenzyme R' (West and Wilson, 1939) and with 'vitamin H' (György *et al.*, 1940). Evidence indicating that biotin is an

essential nutrient for a wide variety of yeast, fungi, bacteria, algae, protozoa, insects, birds and mammals has been reviewed by Briggs (1961) and Langer and György (1968).

B. Biotin in Poultry Nutrition

(i) Chickens

The symptoms of biotin deficiency in chicks were first described by Ringrose, Norris and Heuser (1931) prior to the discovery of the vitamin. The syndrome was characterized mainly by dermatitis and poor feathering. Dermatitis developed around the eyes, at the corners of the beak and on the feet. The margins of the eyelids became granular and constricted, and a viscous exudate was produced, frequently causing the eye-lids to stick together. The crusty scabs at the angle of the beak soon enlarged to include the area around the nostrils and underneath the lower mandible. The skin at the bottom of the feet peeled off and the underlayer thickened and cornified; hemorrhagic cracks then appeared, and wart-like protruberances developed on the balls of the feet. Feather growth was retarded, and the feathers produced were rough and staring.

In addition to symptoms of dermatitis and abnormal feathering, the appearance of perosis has been reported in chicks fed raw egg white (McElroy and Jukes, 1940; Richardson, Hogan and Miller, 1942), and chicks fed biotin-deficient, simplified rations (Jukes and Bird, 1942; Richardson et al., 1942). The deficiency symptoms usually appear at two to four weeks of age (Ringrose et al., 1931; McElroy and Jukes, 1940; Richardson et al., 1942).

Symptoms of a biotin deficiency in chicks have been prevented by adding biotin-rich ingredients or pure biotin to the feed or by

injecting the birds with pure biotin. Feeding sources of biotin such as yeast or milk vitamin concentrate (Ringrose et al., 1931); extracts of yeast, liver and kidney (Hegsted et al., 1940); molasses (Hegsted et al., 1940; McElroy and Jukes, 1940); dried rumen contents from a cow (McElroy and Jukes, 1940) as well as crystalline biotin (Ansbacher and Landy, 1941) prevented symptoms of a biotin deficiency and improved growth rate of chicks. Daily injections of 35 µg of biotin (Hegsted et al., 1940) or 2.5 µg of biotin methyl ester (Ansbacher and Landy, 1941) also prevented the symptoms. Jukes and Bird (1942) observed that a daily average of 0.65 µg of biotin methyl ester (0.34 µg biotin) per chick given in seven injections between the 12th and 32nd day of age was sufficiently high to prevent perosis but was only partially effective in relieving the symptoms of dermatitis. Ansbacher and Landy (1941) and Hegsted et al. (1942) suggested that the minimal curative dose for chicks was approximately 100 µg/kg of ration.

Although symptoms of a biotin deficiency are similar in many ways to those of a pantothenic acid deficiency, some differences have been noted. Hegsted et al. (1940), McElroy and Jukes (1940) and Ansbacher and Landy (1941) observed that, in the case of a biotin deficiency, dermatitis of the feet was quite pronounced before mandibular lesions developed whereas, in a pantothenic acid deficiency the dermatitis of the feet rarely became as severe as in a biotin deficiency. Perosis was not reported to be a common symptom of a pantothenic acid deficiency.

Biotin has also been shown to be necessary for adequate nutrition of breeding chickens. Cravens et al. (1942 and 1944) reported that biotin was essential for reproduction and embryonic development

of the domestic fowl. A deficiency of biotin resulted in decreased hatchability and a high incidence of micromelia and syndactyly in embryos that failed to hatch.

(ii) Turkeys

Symptoms of a biotin deficiency in turkeys are similar to those in chickens. They include dermatitis of the feet, beak angle and eye, perosis, retarded feathering, slow rate of growth and high mortality. As in chickens, symptoms of deficiency appear at two to four weeks of age (Patrick et al., 1941 and 1943). Patrick et al. (1941) fed chicks and poults simplified and commercial-type rations considered to be adequate in all known nutrients except biotin. Both chicks and poults developed symptoms of biotin deficiency when fed the simplified ration but only poults developed deficiency symptoms when fed the commercial-type ration. The deficiency symptoms were prevented by supplementing the rations with liver residue, yeast residue, dried brewer's yeast or a biotin concentrate. The dietary biotin requirement of poults was estimated to be 2.5 times that of chicks with a daily requirement of 2 and 5 µg for chick and poult respectively. Later work by Patrick et al. (1943) showed the effectiveness of biotin as an antiperotic factor in the poults' diet.

Symptoms of a deficiency in poults similar to those described for a biotin deficiency by Patrick et al. (1941 and 1943) were produced in poults by McGinnis and Carver (1947) who fed a ration composed of natural ingredients but low in riboflavin. The effect of supplemental riboflavin and biotin on occurrence of the syndrome was studied. Biotin was not found to be essential for the prevention of dermatitis but, in some trials, it was needed in addition to riboflavin to prevent perosis.

Riboflavin was effective in preventing dermatitis, perosis and poor feathering and promoting growth when added to a basal diet containing a sulfa drug. It was suggested that riboflavin was used directly by the poults for these functions and did not exert its effect by causing bacterial synthesis of some other factor in the intestinal tract.

The occurrence of symptoms of a biotin deficiency in poults fed practical rations have also been reported by Robblee and Clandinin (1953). It was noted that the symptoms of the condition became more severe as the hatching season progressed. The disorder was alleviated to some extent in early hatched poults by the addition of calcium pantothenate to the rations, but in later hatched poults calcium pantothenate was relatively ineffective. A combination of biotin and calcium pantothenate was effective in alleviating the disorder in late hatched poults. Of the two vitamins, biotin was the more critical. The need for biotin in practical poult rations was confirmed by Slinger and Pepper (1954) who also observed that the requirements for biotin were reduced by the inclusion of antibiotics in the feed. A ration containing 280 µg of biotin per kg, and which supplied 8.4 µg of biotin per poult per day, was insufficient to meet the requirement for the vitamin in the absence of antibiotics.

Recently, the appearance of symptoms in turkey flocks similar to those described by Robblee and Clandinin (1953) have been reported in the U.S.A. (Misner, 1967; Richardson and Wilgus, 1967; Waibel *et al.*, 1967; Johnson, 1967; Jensen and Martinson, 1969). Waibel *et al.* (1967) have suggested biotin requirements of 208 and 187 µg/kg for prestarter and starter rations for turkeys respectively. This was supported in a later experiment (Waibel *et al.*, 1969) in which 193 µg of biotin

per kg of prestarter poults' ration was found to be adequate to meet the poults' requirements for the vitamin. Scott (1968) suggested that a level of 225 µg of biotin per kg of ration was adequate for the poult. Jensen and Martinson (1969) reported that 284 µg of biotin per kg of ration gave optimal growth of poults in one experiment but in a second experiment a higher level of biotin appeared to be necessary.

C. Factors Affecting Estimation of the Biotin Requirements of Poultry

There are many factors which may influence the estimation of the biotin requirements of poultry. One of the most important of these is the availability of biotin in the feed ingredients that comprise the ration. Patrick et al. (1942) suggested that it was possible for practical turkey starter rations to be deficient in available biotin since all naturally-occurring biotin in feedstuffs is not in available form. The availability of biotin in hulless barley and barley was found by Wagstaff, Dobson and Anderson (1961) to be about one-third of the total biotin content. Microbiological assays showed that hulless barley and barley contained 0.10 and 0.13 µg of biotin per gram respectively, while a biological assay using the chick indicated a biotin content of only 0.035 µg/g in each case. The quantity of biotin available in feed ingredients may also be affected by the wide variation in biotin content within and between feed ingredients (National Research Council, 1964).

Another factor which may affect the amount of biotin available to the bird is the stability of biotin in mixed rations. Pavcek and Shull (1942) reported that biotin was inactivated in vitro by rancidified fat or pure ethyl linoleate. Ethyl linoleate inactivated 96% of pure biotin in 12 hours and 99% in 48 hours, but in the presence of Vitamin E 60% of the biotin remained after 48 hours. Thus the possibility exists

that rancidity in feeds might affect the level of biotin contained therein.

The need for dietary biotin may also be influenced by intestinal synthesis of the vitamin, and this in turn may be influenced by the type of carbohydrate in the diet. Couch et al. (1948) and Couch et al. (1949) observed that use of dextrin in the diet of the laying hen favoured intestinal synthesis of biotin whereas use of sucrose did not. The observation was supported by Johansson, Sarles and Shapiro (1948) who found that when dextrin was fed as a main carbohydrate source, the microflora of the gut of the laying hen contained a predominance of biotin-synthesizing organisms but when sucrose was fed there were more biotin-utilizing organisms present, including large numbers of yeast in the ceca. Competition between intestinal microflora and the host animal for biotin was suggested by Sunde et al. (1950) who found that cecectomized hens laid eggs with higher biotin content and better hatchability than normal hens fed the same ration. It was also suggested that microorganisms such as yeast, which are high biotin absorbers (Peterson, 1948), develop in the cecum and may be responsible for reducing the biotin available to the bird.

The presence of drugs in the diet may also affect the dietary requirement for biotin by enhancing or retarding intestinal synthesis of the vitamin. Slinger and Pepper (1954) found that the addition of 15 mg of penicillin or oxytetracycline per kg of ration spared the biotin requirements of poults. Chow, Davis and Davis (1953) reported that moderately high levels of dietary chlortetracycline depressed free biotin in the feces of rats fed a semi-purified ration, while streptomycin, penicillin and bacitracin caused an increase in apparent intestinal synthesis.

The presence of antimetabolites which may increase specific requirements for biotin was hypothesized by Richardson and Wilgus (1967). It was suggested that antimetabolites may occur naturally in feeds or may be present as drugs, other additives, or as contaminants. It was also suggested that the ability of the gut to absorb nutrients may be impaired by intestinal infections.

There is some evidence that other vitamins in rations may alter the requirements for biotin. The inclusion of riboflavin in the ration was observed by Nielsen and Elvehjem (1942) to exert a favourable action on the synthesis of biotin in the intestinal tract of the rat although no biotin-sparing action was detected. Pyridoxine, according to MacKay and Barnes (1941), reduced the severity of biotin deficiency symptoms in rats, but no protective effect was found in subsequent studies by Nielsen and Elvehjem (1942), and Johnson, Jensen and Parsons (1952). Ascorbic acid and vitamin B_{12} have also been shown to exert a temporary biotin-sparing effect by Terroine (1954) and Marchetti and Testoni (1964) respectively. Terroine (1954) observed that the addition of ascorbic acid to biotin-free diets increase appetite, prevented apparent deficiency symptoms and prolonged survival time in the rat. Terroine (1960) later suggested that ascorbic acid prevented only those processes leading to characteristic deficiency symptoms but not those causing death in a biotin-deficient state. Marchetti and Testoni (1964) showed that the addition of 100 ug of vitamin B_{12} per kg of biotin-deficient ration promoted growth in the rat and delayed the onset of classical biotin-deficiency symptoms.

Bauernfeind (1969) suggested that the interrelationships of biotin with other vitamins involved in protein, carbohydrate and lipid metabolism may influence requirements for biotin. It was also suggested

that there is need for greater consideration of the influence of efficiency of feed conversion and of environmental and genetic factors on the nutrient requirements of poultry.

D. Determination of Biotin by Biological Assays

The presence of biotin in trace amounts in foods, concentrates, animal and plant tissues excludes the use of direct chemical and physical methods for assay purposes, and necessitate the use of microbiological or animal assays.

Microbiological assays involving the use of either Lactobacillus plantarum ATCC 8014 or Lactobacillus casei are the most widely used methods for biotin assay. Shull and Peterson (1943) used L. casei, but Wright and Skeggs (1944) were more successful using L. plantarum.

Microbiological assays have the advantages of being inexpensive, technically simple, short term procedures, permitting the simultaneous assay of a large number of samples. Concentrations as low as 0.2 mμg/ml of solution or 10 μg/kg of feed can be readily detected.

The short-comings of the microbiological method are mainly due to interference from substances which may be present in the assay medium. These may have, in varying degrees, stimulating or inhibitory effects on the test organism. Stimulation of growth of biotin test organisms by oxybiotin (Pilgrim et al., 1945), N-biotinyl glycine (Wright, Skeggs and Cresson, 1951), biotin-d-sulfoxide (Melville, Genghof and Lee, 1954) and oleic acid in the presence of aspartic acid (Broquist and Snell, 1951) has been reported. Fortunately, the biotin derivatives do not occur in significant quantities in natural materials, and oleic acid may be removed by washing with peroxide-free

diethyl ether (Association of Vitamin Chemists, 1966). Lysolecithin (Trager, 1948) has a highly inhibitory effect on the growth of lactic acid bacteria in the presence of sub-optimal levels of biotin. For this reason removal of this compound from assay materials is necessary.

Choice of a test organism for assay purposes has been influenced by the nature of the investigation. Because L. casei is capable of using derivatives of biotin in place of biotin for growth, it is useful in studies involving naturally-occurring biotin derivatives. L. plantarum however, has been shown to be highly specific in its response to free biotin and hence the Association of Vitamin Chemists (1966) recommended it as the best organism for the microbiological assay of biotin.

Animal assays for biotin have also been developed. Factors which tend to complicate microbiological assays are eliminated in animal assays, as in the case of a chick assay for biotin (György, 1951). However, the long duration, large numbers of chicks required, battery space, expense of purified diets and labour requirements militate strongly against animal assays. Nevertheless, animal assays remain the only method for determining the amount of biotin in a feed that is available to the chicken or turkey.

EXPERIMENTS AT THE UNIVERSITY OF ALBERTA

Experiments were designed to study:

I. The biotin requirements of turkey poults.

II. The biotin content of feed ingredients and commercial turkey starter rations used in Alberta.

III. The effects of progression of the hatching season on the biotin content of yolks of turkey eggs and livers of day-old poults.

IV. The effects of dietary biotin level and ration type on storage of biotin in livers of 4-week-old poults.

I. The Biotin Requirements of Turkey Poults.

Status of the Problem

The reappearance in the past few years of a disorder in poults attributed to a biotin deficiency has resulted in heavy losses by turkey producers in Alberta. Recommendations for both prophylactic and therapeutic treatments have been made, yet there is little up-to-date information available on the biotin requirements of poults.

In order to assess the biotin requirements of poults it seemed desirable that a purified ration, low in biotin content, be used. Since there had been no reports of a suitable purified ration for turkey poults, an experiment was commenced to try to develop a purified ration for assessing the biotin requirements of poults.

Experimental (General)

Day-old, Broad-Breasted White turkey poults of mixed sexes from the University of Alberta Research Farm were used in all feeding trials. The poults were individually weighed, wingbanded and randomly alloted to give comparable groups of eight to twelve poults. The poults were reared in electrically heated batteries with raised screen floors. Experimental rations and water were supplied ad lib.

Individual body weights and mortality were recorded at weekly intervals, at which time the poults were scored for foot-pad dermatitis, hock disorder, broken feathers, dermatitis of the beak and eye, and diarrhoea. The trials were terminated when the poults were 4 weeks old. Three trials were conducted during 1968 and three during 1969.

Statistical Analysis

Analysis of variance was performed on the data by an IBM 360 model 67 computer at the University of Alberta using the procedure

outlined by Smillie (1969). Significant mean differences among treatment groups were assessed using Duncan's new multiple range test (Steel and Torrie, 1960).

Trial 1

Object

To devise a suitable purified ration for studies of the biotin requirements of turkey poults.

Experimental

The formulation of experimental rations used were patterned after purified rations used for chickens but the levels of protein, minerals and vitamins were increased to more closely approximate the requirements of turkeys (National Research Council, 1966). In the trial 64 poults were divided into 8 groups of 8 poults each. The treatments were arranged in a 2 X 2 X 2 factorial design involving two protein sources (gelatin - vitamin-free casein - isolated soybean protein and isolated soybean protein), two levels of protein (28 and 33.3%) and two levels of biotin (0 and 500 µg/kg of ration). The composition of rations fed is shown in Table 1.

Results and Discussion

A summary of the effects of treatment on average body weight, percentage mortality and deficiency symptoms of poults at 28 days of age are presented in Table 2.

A deficiency of biotin (Groups 1,3,5 and 7) resulted in a low rate of growth and a high level of mortality. Hock disorder, dermatitis and broken feathers were noted in all poults that survived to 4 weeks of age. Symptoms of dermatitis were very severe. Scabby encrustations were present at the beak angles and around the eyes; foot-pads were thickened

Table 1. Compositions of purified rations.

Ingredients	Ration Number							
	1 %	2 %	3 %	4 %	5 %	6 %	7 %	8 %
Cerelose	55	55	49	49	54.8	54.8	48.8	48.8
Corn Oil	5	5	5	5	5	5	5	5
Gelatin	10	10	10	10	-	-	-	-
Vit-free Casein	10	10	10	10	-	-	-	-
Isolated Soy Protein	11	11	17	17	31	31	37	37
Mineral Mix[1]	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
$CaCO_3$	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
$CaHPO_4 \cdot 2H_2O$	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7
NaCl	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6
KCl	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin Mix[2]	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Choline Chloride (pure)	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
D-L Methionine	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
D-L Tryptophan	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Glycine	-	-	-	-	0.2	0.2	0.2	0.2
Folic Acid Premix[3]	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Biotin[4]		+		+		+		+

[1]Mineral mix added per kg of ration consisted of: KH_2PO_4, 9.3 g; $MgSO_4$, 4.9 g; KI, 0.0029 g; $FeSO_4 \cdot 7H_2O$, 0.28 g; $ZnCO_3$, 0.125 g; $CoCl_2 \cdot 6H_2O$, 0.0017 g; Na_2MoO_4 $2H_2O$, 0.0093 g; Na_2SeO_3, 0.00022 g; $MnSO_4 \cdot H_2O$, 0.37 g; $CuSO_4 \cdot 5H_2O$, 0.028 g.

[2]Vitamin mix added per kg of ration consisted of: Vitamin A, 10,000 I.U.; vitamin D_3, 1,800 I.C.U.; vitamin E, 20 I.U.; menadione, 2 mg; thiamine hydrochloride, 10 mg; riboflavin, 6 mg; niacin, 100 mg; calcium pantothenate, 20 mg; pyridoxine hydrochloride, 6 mg; vitamin B_{12}, 0.03 mg; ascorbic acid 45 mg.

[3]Folic acid premix at the rate of 0.1% supplied 5 mg of folic acid per kg of ration.

[4]Biotin additions were made at the level of 500 µg/kg of ration.

Table 2. Effect of protein source, protein level and biotin supply in the ration on performance of poults.

Group No.	Protein Supplement*	Ration Protein Content %	Biotin Supplement (µg/kg)	Observations at 4 Wk of Age: Avg Body Wt (g)	Mortality %	Hock Disorder %	Dermatitis: Beak and Eye %	Dermatitis: Feet %	Broken Feathers %
1	Gel-Cas-Soy	28	0	176	87.5	100	100	100	100
2	Gel-Cas-Soy	28	500	435	50	25	0	0	25
3	Gel-Cas-Soy	33.3	0	135	87.5	100	100	100	100
4	Gel-Cas-Soy	33.3	500	511	0	12.5	25	0	12.5
5	Soy	28	0	196	37.5	100	100	100	100
6	Soy	28	500	421	0	12.5	12.5	0	0
7	Soy	33.3	0	216	25	100	100	100	100
8	Soy	33.3	500	418	0	0	12.5	0	0

*Gel-Cas-Soy refers to a mixture of gelatin, vitamin-free casein and isolated soybean protein.

and hemorrhagic cracks occurred on the feet of many of the birds. Growth of feathers was retarded and many were broken giving the birds a rough ragged appearance.

The addition of biotin to the rations (Groups 2,4,6 and 8) resulted in increased rate of growth, reduced mortality and considerable alleviation of the deficiency symptoms. The use of biotin however, did not completely eliminate the symptoms of deficiency.

Source and level of protein used had some effect on the performance of poults. In the biotin-deficient groups, the use of the mixture of protein gave lower rate of growth and higher mortality than the use of isolated soybean protein alone. This difference may be attributed to a higher level of biotin in isolated soybean protein than in gelatin and vitamin-free casein. In the biotin supplemented groups, rate of growth was highest in the poults receiving a high protein ration containing a mixture of protein sources (Group 4).

Although the performance of the poults fed purified rations containing biotin was reasonably satisfactory, the rate of growth achieved was somewhat lower than that of poults raised on high quality practical rations in other experiments.

Summary

Purified rations containing combinations of two sources of protein (gelatin - vitamin-free casein - isolated soybean mixture and isolated soybean protein alone), two levels of protein (28 and 33.3%) and two levels of biotin (0 and 500 µg/kg were fed to poults from 0 to 4 weeks of age. The results obtained indicated the following:

(1) When biotin was not included in the rations severe symptoms of a biotin deficiency resulted.

(2) Supplementing the rations with biotin improved rate of growth and almost eliminated the symptoms of biotin deficiency.

(3) In biotin-supplemented rations, a level of 33.3% protein resulted in a higher rate of growth than 28% protein.

(4) Use of a mixture of gelatin, vitamin-free casein and isolated soybean protein in the ration appeared to be superior to isolated soybean protein alone, because deficiency symptoms were more severe when biotin was omitted and rate of growth was superior to other treatments when biotin was included in the ration.

Trials 2 and 3

Object

To study the effect of levels of biotin supplementation in a purified ration on rate of growth and performance of poults.

Experimental

Fifty-six poults, divided into 7 groups of 8 poults each, were employed in each trial. The purified basal ration used was the same as Ration 3, Table 1. Levels of biotin supplementation used were: 0, 50, 100, 150, 200, 300 and 500 µg/kg of ration.

Results and Discussion

The effects of treatment on average body weight, percentage mortality and deficiency symptoms of poults at 28 days of age are presented in Table 3.

In Trial 2, as the level of biotin supplementation increased, rate of growth tended to increase and percentage mortality decreased until a level of 300 µg/kg of ration (Group 6) was reached. Increasing

Table 3. Effect of level of biotin added to a purified ration on performance of poults.

Group No.	Biotin Supplement (µg/kg)	Observations at 4 Wk of Age						
		Avg Body Wt (g)	Mortality %	Hock Disorder %	Dermatitis		Broken Feathers %	Diarrhoea %
					Beak and Eye %	Feet %		
Trial 2								
1	0	175	75	100	100	100	100	100
2	50	216	37.5	80	100	60	100	100
3	100	472	12.5	43	43	14	86	14
4	150	412	12.5	57	86	29	86	0
5	200	512	12.5	14	29	14	43	0
6	300	517	0	0	0	0	0	0
7	500	421	37.5	0	0	0	0	0
Trial 3								
1	0	216	87.5	100	100	100	100	0
2	50	324	50	100	75	75	100	0
3	100	463	12.5	0	0	0	0	0
4	150	354	12.5	0	0	0	100	0
5	200	433	12.5	0	0	0	100	0
6	300	471	0	0	0	0	100	0
7	500	453	0	0	0	0	0	0

the level to 500 µg/kg of ration (Group 7) resulted in a lower average body weight and a higher level of mortality than was obtained with 300 µg/kg.

Symptoms of biotin deficiency were severe in groups receiving 0 and 50 µg of biotin per kg of ration (Groups 1 and 2), but lessened as the level of biotin supplementation increased to 300 µg/kg of ration. Poults receiving this level of supplementation (Group 6) showed no symptoms of disorder and were generally comparable to those raised on good quality practical rations. With the higher level of supplementation (500 µg/kg) no symptoms of biotin deficiency were noted.

In Trial 3 the results obtained were much more variable than in Trial 2. As in the previous trial, poults receiving levels of 0 and 50 µg of biotin per kg of ration showed severe symptoms of biotin deficiency (Groups 1 and 2). Addition of 100 µg of biotin per kg of ration (Group 3) resulted in rapid growth and complete absence of deficiency symptoms. Higher levels of biotin supplementation (150, 200 and 300 µg/kg) in the ration failed to increase rate of growth above that obtained with a supplementation of 100 µg of biotin per kg. In addition, all poults in the groups showed severe breaking of feathers (Groups 4, 5 and 6). A level of 500 µg of biotin per kg of ration (Group 7) completely prevented symptoms of a biotin deficiency.

Summary

Two feeding trials were conducted to determine the biotin requirements of poults. Graded levels of biotin (0, 50, 100, 150, 200, 300 and 500 µg/kg) were fed in purified rations from 0 to 4 weeks of age.

The results obtained were variable. In Trial 2 a level of 300 µg of biotin per kg of ration appeared to meet the requirements of

the poult. In Trial 3 satisfactory rate of growth and freedom from deficiency symptoms were obtained with 100 µg of biotin per kg of ration but some deficiency symptoms were noted with higher levels of supplementation (150, 200 and 300 µg/kg). No deficiency symptoms were noted in poults receiving 500 µg of biotin per kg of ration.

Trial 4

Object

It had been noted previously (see "Review of Literature") that biotin requirements were modified by the inclusion of an antibiotic in the ration. This trial was conducted to determine whether inclusion of chlortetracycline in a purified ration would affect the poults requirement for biotin.

Experimental

One hundred and sixty poults were divided into 20 comparable groups of 8 birds each and fed the experimental rations. Each ration was fed to two groups of poults. The basal ration used was the same as that used in Trials 2 and 3. Five levels of biotin (0, 150, 225, 300 and 400 µg/kg of ration) in combination with two levels of chlortetracycline (0 and 50 mg/kg of ration) were used as experimental treatments.

Results and Discussion

A summary of the effects of treatment on average body weight, percentage mortality and occurrence of deficiency symptoms at 28 days of age are presented in Table 4.

A deficiency of biotin (Groups 1 and 2) resulted in a low rate of growth and a high rate of mortality. When the level of biotin in the ration was increased to 150 µg/kg of ration (Groups 3 and 4) a significant increase in growth rate and a decrease in rate of mortality

Table 4. Effect of levels of biotin and chlortetracycline in a purified ration on performance of poults.

Group No.	Biotin Supplement (µg/kg)	Aureomycin Supplement (mg/kg)	Observations at 4 Wk of Age						
			Avg Body Wt (g)	Mortality %	Hock Disorder %	Dermatitis		Broken Feathers %	Diarrhoea %
						Beak and Eye %	Feet %		
1	0	-	165	87.5	100	0	100	100	100
2	0	50	162	87.5	100	0	100	100	100
3	150	-	452	18.7	77	0	0	100	0
4	150	50	504	6	12.5	21.5	0	50	0
5	225	-	547	0	0	0	0	0	0
6	225	50	520	18.7	0	0	0	0	0
7	300	-	496	6	0	0	0	0	0
8	300	50	549	0	0	0	0	0	0
9	400	-	478	6	0	0	0	0	0
10	400	50	462	0	0	0	0	0	0

occurred. Maximum rate of growth was achieved with a biotin level in the ration of 225 µg/kg (Groups 5 and 6).

Symptoms of biotin deficiency were severe in the groups receiving 0 and 150 µg of biotin per kg of ration (Groups 1 to 4). Higher levels of biotin (225, 300 and 400 µg/kg) in the rations completely prevented all deficiency symptoms. In this trial symptoms of dermatitis of the beak and eye were absent in all groups except group 4.

The addition of chlortetracycline to the rations did not have any significant effect ($P < 0.05$) on rate of growth although it lessened the severity of deficiency symptoms when the level of biotin in the ration was marginal (Group 4 vs Group 3). No significant interaction ($P < 0.05$) between levels of biotin and chlortetracycline on rate of growth was observed.

Summary

Poults were fed rations containing five levels of biotin (0, 150, 225, 300 and 400 µg/kg of ration) in combination with two levels of chlortetracycline (0 and 50 mg/kg of ration), from 0 to 4 weeks of age. The results obtained indicated:

(1) A level of 225 µg of biotin per kg of ration appeared to meet the poults requirement for biotin to 4 weeks of age.

(2) The addition of chlortetracycline to the purified ration did not influence the dietary requirement of the poult and apparently had no effect on rate of growth. The inclusion of chlortetracycline did appear, however, to lessen the severity of deficiency symptoms when the level of biotin in the ration was marginal.

Trial 5

Object

The previous trials indicated that the purified ration used, when supplemented with sufficient biotin, permitted relatively good rate of growth and freedom from symptoms of biotin deficiency. Rate of growth, however, was not comparable to that usually obtained when good quality, practical-type rations were fed. It therefore seemed possible that the purified ration was lacking in an unidentified growth factor (UGF) or that the level of nutrients supplied was insufficient to allow optimal performance. About that time, Sullivan, Heil and Armintrout (1967) reported the composition of a purified ration that had been used in some experiments with turkeys. The ration differed from the one used here (Ration 3, Table 1) in that isolated soybean protein was used as the source of protein and levels of vitamins added were considerably higher. Consequently, a trial was undertaken to determine whether protein source, vitamin levels or inclusion of sources of UGF in purified rations would affect rate of growth and general performance of turkey poults.

Experimental

Sixty-four poults, divided into 8 comparable groups of 8 poults each, were used. The trial was designed as a 2 X 2 X 2 factorial involving two sources of protein, two levels of vitamin supplementation and the absence and addition of sources of UGF in the rations. All rations contained biotin at the level of 300 µg/kg. The compositions of the rations fed and the vitamin mixtures used are shown in Tables 5 and 6.

Results and Discussion

The effect of treatment on average body weight of poults at 4

Table 5. Compositions of rations.

Ingredients	Ration Number							
	1 %	2 %	3 %	4 %	5 %	6 %	7 %	8 %
Cerelose	48.15	43.65	48.05	43.55	44.35	39.85	44.25	39.75
Solka Floc	-	-	-	-	4	4	4	4
Corn Oil	5	5	5	5	5	5	5	5
Gelatin	10	10	10	10	-	-	-	-
Vitamin Free Casein	10	10	10	10	-	-	-	-
Isolated Soy Protein	17	15.5	17	15.5	37	35.5	37	35.5
Brewers Yeast	-	2	-	2	-	2	-	2
Distillers Solubles	-	2	-	2	-	2	-	2
Dried Whey	-	2	-	2	-	2	-	2
Mineral Mix[1]	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
$CaCO_3$	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
$CaHPO_4 \cdot 2H_2O$	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2
NaCl	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6
KCl	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin Mix 1[2]	0.4	0.4	-	-	0.4	0.4	-	-
Vitamin Mix 2[2]	-	-	0.4	0.4	-	-	0.4	0.4
Choline Chloride (pure)	0.3	0.3	0.4	0.4	0.3	0.3	0.4	0.4
DL Methionine	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
DL Tryptophan	0.2	0.2	0.2	0.2	-	-	-	-
Glycine	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Furazolidone	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2

[1]Same as shown in Table 1.

[2]See Table 6.

Table 6. Composition of vitamin mixtures.*

Ingredient		Vitamin Mix 1	Vitamin Mix 2
Vitamin A	(I.U.)	10,000	15,000
Vitamin D_3	(I.C.U.)	1,800	3,000
Vitamin E	(I.U.)	20	44
Menadione	(mg)	2	4
Thiamine hydrochloride	(mg)	10	15
Riboflavin	(mg)	6	10
Niacin	(mg)	100	100
Calcium pantothenate	(mg)	20	30
Pyridoxine hydrochloride	(mg)	6	8
Folic acid	(mg)	5	6
Biotin	(mg)	0.3	0.3
Vitamin B_{12}	(mg)	0.03	0.06
Ascorbic acid	(mg)	45	45
Inositol	(mg)	-	1,000

* Levels added per kg of ration.

weeks of age is shown in Table 7. No symptoms of a biotin deficiency occurred in any of the groups.

Analysis of variance of the data indicated that significant differences ($P < 0.01$) existed between treatments. As had been noted previously (Trial 1) rations containing gelatin, vitamin-free casein and isolated soybean protein (Groups 1, 2, 3 and 4) gave a higher rate of growth than rations containing isolated soybean protein alone (Groups 5, 6, 7 and 8). Average body weight at 4 weeks of age was not affected by the levels of vitamins supplied in the rations. Inclusion of brewer's yeast, distiller's solubles and dried whey in the rations as sources of UGF activity resulted in a significant ($P < 0.05$) increase in body weights at 4 weeks of age.

Despite the fact that rate of growth was superior when the mixed protein was used, visual examination of the birds indicated that

Table 7. Influence of source of protein, level of vitamins and addition of sources of UGF[1] on growth of poults.

Group No.	Ration Formulation Used[2]	Vitamin Mix[3] Used	Added UGF	Avg Body Wt at 4 Wk of Age (g)
1	1	1	-	575
2	1	1	+	636
3	1	2	-	572
4	1	2	+	670
5	2	1	-	477
6	2	1	+	536
7	2	2	-	566
8	2	2	+	561

[1]UGF = 2% brewer's yeast + 2% distiller's solubles + 2% dried whey.

[2]1 represents rations containing gelatin, vitamin-free casein and isolated soybean protein as sources of protein.

2 represents rations containing isolated soybean protein as the only protein source.

[3]See Table 6.

feathering and general appearance of the groups receiving isolated soybean protein and the higher levels of vitamins (Groups 7 and 8) were superior to that of other groups.

Summary

Poults were fed purified rations containing two protein sources and two levels of vitamin supplementation with and without added sources of UGF activity to 4 weeks of age. Visual examination and the average body weights obtained at this time indicated that:

(1) Feeding purified rations containing a mixture of gelatin, vitamin-free casein and isolated soybean protein as the source of protein resulted in a significantly higher rate of growth than feeding similar rations containing isolated soybean protein as the only protein source.

(2) Levels of supplemental vitamins used had no effect on the rate of growth or performance of poults.

(3) The inclusion in the rations of brewer's yeast, distiller's solubles and dried whey as sources of UGF resulted in a significant increase in the rate of growth of poults as compared to unsupplemented groups.

(4) Feathering and general appearance of poults fed the rations containing isolated soybean protein as the only protein source and higher levels of vitamins were superior to other poults in the experiment.

Trial 6

Object

To study the effects of graded levels of biotin in purified rations, with and without sources of UGF activity, on the performance of poults.

Experimental

One hundred and twenty poults were divided into 10 comparable groups of 12 poults each and fed the experimental rations. The treatments imposed included feeding five levels of supplemental biotin (0, 100, 200, 300 and 400 µg/kg of ration) in rations with or without the addition of brewer's yeast, distiller's solubles and dried whey as sources of UGF. The basal ration was the same as ration 7, Trial 5. Additions of brewer's yeast, distiller's solubles and dried whey at a level of 2% each were made at the expense of 4.5% cerelose and 1.5% isolated soybean protein.

Results and Discussion

The effects of treatment on average body weight, mortality rate and occurrence of deficiency symptoms at 28 days of age are presented in Table 8.

A deficiency of biotin (Groups 1 and 2) resulted in reduced rate of growth and severe symptoms of deficiency. The addition of 100 µg of biotin per kg of ration greatly reduced deficiency symptoms and caused a significant increase in rate of growth (Groups 3 and 4) but addition of 200 µg of biotin per kg was required to eliminate deficiency symptoms when sources of UGF were not included (Group 3). The addition of greater amounts of biotin (200, 300 and 400 µg/kg) did not improve rate of growth but completely prevented the symptoms of biotin deficiency.

The inclusion in the ration of UGF resulted in a significantly higher ($P < 0.05$) rate of growth at all levels of biotin supplementation. When biotin was not added (Group 2) rate of growth was low and severe symptoms of a biotin deficiency were noted; however, the addition of 100 µg of biotin per kg of ration was sufficient to permit optimum growth and complete absence of symptoms of a biotin deficiency.

Table 8. Effects of biotin supply and UGF on performance of poults.

Group No.	Biotin Supplement (µg/kg)	Added UGF	Observations at 4 Wk of Age						
			Avg Body Wt (g)	Mortality %	Hock Disorder %	Dermatitis Beak and Eye %	Dermatitis Feet %	Broken Feathers %	Diarrhoea %
1	0	-	292	8.3	100	91	91	100	63.6
2	0	+	426	8.3	100	82	63.6	100	18.2
3	100	-	530	8.3	9	0	0	0	0
4	100	+	604	16.7	0	0	0	0	0
5	200	-	482	8.3	0	0	0	0	0
6	200	+	545	0	0	0	0	0	0
7	300	-	510	16.7	0	0	0	0	0
8	300	+	549	8.3	0	0	0	0	0
9	400	-	521	8.3	0	0	0	0	0
10	400	+	561	25	0	0	0	0	0

Summary

Poults were fed purified rations containing five levels of biotin supplementation with and without sources of UGF activity. The results obtained indicated:

(1) The addition of 200 µg of biotin per kg of purified ration appeared to meet the requirements of poults for growth and prevention of deficiency symptoms, while the addition of 100 µg of biotin per kg was sufficient when sources of UGF were included in the ration.

(2) The inclusion of sources of UGF in the ration resulted in a significantly higher rate of growth regardless of the level of biotin supplementation used.

II. The Biotin Content of Feed Ingredients and Commerical Rations used in Alberta.

Status of the Problem

During the past twenty years sporadic occurrences of a biotin deficiency syndrome have been noted in commercial turkey flocks in Alberta but until recently reports of a similar disorder elsewhere have not appeared. This suggested the possibility that the biotin content of feed ingredients in use in Alberta might be lower than that found in ingredients used elsewhere. Consequently an experiment was undertaken to determine the biotin content of feed ingredients commonly used in Alberta and to ascertain the biotin levels in some commercial feeds produced in this area.

Experimental

Samples of 15 feed ingredients and of 6 commercial feeds were assembled for determination of their biotin content. The samples were finely ground and stored in a refrigerator until the assays were completed.

For the determinations 2 to 5 grams of each feed ingredient and commercial feed were hydrolysed with 50 ml of 4 N H_2SO_4 for 2 hours in an autoclave at 15 lb pressure. The hydrolysate was cooled and filtered through No 2 Whatman filter paper. The filtrate was washed twice with 20 ml of peroxide-free diethyl ether and was diluted to 200 ml. An aliquot was taken, neutralized to pH 6.8 and diluted to volume with distilled water. The final dilution used was within the range of the limits of the assay procedure. The biotin content of the hydrolysates were then determined using the microbiological assay technique (Association of Vitamin Chemists, 1966).

Results and Discussion

The levels of biotin found in the feed ingredients and commercial

Table 9. Biotin content of feed ingredients and commercial turkey starter rations used in Alberta.

Ingredient	Biotin content from present microbiological assay (µg/kg)	Reported Values of Biotin Content				
		NRC[1] (µg/kg)	Md (61)[2] (µg/kg)	W.S.U.[3] (µg/kg)	Mü[4] (µg/kg)	Roche[5] (µg/kg)
Wheat	75	110	110	90	90	110
Oats	146	300	300	-	180	110
Barley	103	190	170	197	130	110
Wheat Shorts	332	-	-	-	-	-
Wheat Bran	453	-	110	-	110	480
Dehydrated Alfalfa Meal	365	-	-	354	135	-
Meat Meal	218	-	90	-	-	260
Herring Meal	446	-	130	150	-	420
Soybean Meal (44%)	446	-	330	248	-	-
Rapeseed Meal	904	-	-	-	-	-
Distiller's Solubles	535	-	-	1180	-	-
Brewer's Yeast	1400	-	1600	750	970	-
Dried Whey	240	390	370	-	-	-
Isolated Soybean Protein	140	-	-	-	-	-
Vitamin-free Casein	17	-	-	-	-	-
Commercial Turkey Starters						
A	465					
B	574					
C	353					
D	458					
E	774					
F	504					

[1]National Academy of Sciences - National Research Council Publication, 1232, 1964. Joint U.S. - Canadian Tables of feed composition.
[2]Combs, G.F. and H. Nott, 1967. Improved nutrient composition data of feed ingredients; amino acids and other nutrient specifications for linear programming of broiler rations. Feedstuffs, Oct. 21, pp. 36-37.
[3]Jensen, L.S. 1967. Biotin in practical turkey rations. Feedstuffs 40(13):18. (1968).
[4]Kirchgessner, M. and H. Friesecke: « Wirkstoffe in der praktischen Tiereernahrung », Bayerischer Landwirtschaftsverlag, Munchen, Basel, Wien. 1966.
[5]Roche - News and Reviews, 1968. p. 10. A publication of Hoffman La Roche Limited.

rations are shown in Table 9. In addition, values found for the same ingredients by other workers have been listed.

Grains, isolated soybean protein and vitamin-free casein were found to be low in biotin content (17 - 146 µg/kg); wheat shorts, wheat bran, dehydrated alfalfa meal, meat meal, herring meal, soybean meal and dried whey contained a medium level of biotin (240 to 453 µg/kg), while distiller's solubles, rapeseed meal and brewer's yeast were relatively high in biotin content (535 to 1400 µg/kg). With such wide variations between feed ingredients it would be possible to formulate rations with biotin content insufficient to meet the biotin requirements of the poult. This, coupled with the likelihood that a portion of the biotin in feedstuffs is unavailable to the poult may account in part at least for the sporadic appearance of biotin deficiency symptoms in the field.

The average biotin content of six turkey starter rations assayed ranged from 353 to 774 µg/kg. Judging from the results of the feeding trials, the commercial rations should contain enough biotin to meet the requirement of the poult. Since all the commercial feeds were supplemented with biotin at levels ranging from 220 to 660 µg/kg, it is obvious that if biotin were not added, the rations could be borderline with respect to the requirement of the poult for biotin.

Summary

The biotin content of 15 feed ingredients and of 6 commercial turkey starter rations were determined by microbiological assay. The average values obtained indicated:

(1) Grains, isolated soybean protein and vitamin-free casein are low in biotin content; wheat shorts, wheat bran,

dehydrated alfalfa meal, meat meal, herring meal, soybean meal and dried whey are of medium biotin content; and distiller's solubles, rapeseed meal and brewer's yeast contain relatively high levels of biotin.

(2) The levels of biotin that were found in commercial rations appeared to be sufficient to meet the requirements of the poult for biotin.

III. The Effects of Progression of the Hatching Season on the Biotin Content of Yolks of Turkey Eggs and Livers of Day-old Poults.

Status of the Problem

The observation, that increased incidence and severity of symptoms of a biotin deficiency occurred (Robblee and Clandinin, 1953) in late hatched poults when the ration was not supplemented with biotin, suggested that the dam's body stores of biotin may decrease as the hatching season progresses. This led to the hypothesis that low body stores in the dam might be reflected in decreased biotin content of eggs produced and reduced biotin levels in the liver of the newly hatched poult. This, in turn, might be responsible for the increased incidence of symptoms of a biotin deficiency in poults late in the hatching season.

In order to ascertain whether the above hypothesis was valid, the biotin content of yolks of turkey eggs and livers of day-old poults, taken at 28 day intervals during the hatching season, was determined.

Experimental

At 28-day intervals throughout the hatching season, two lots of 6 eggs each were taken from the turkey breeding flock at the University of Alberta Poultry Research Farm, and the yolks were assayed for biotin content. In addition, two lots of 6 poults each were taken from day-old poults hatched from eggs produced at the same time as those used for biotin assay and their livers were removed for biotin determination. From each lot of 6 eggs the yolks were separated, pooled, homogenized and stored at -20 C; the livers from each lot of poults were also homogenized and stored at -20 C until assayed for biotin content.

Representative samples of egg yolks and livers were hydrolysed with 50 ml of 4 N H_2SO_4 in an autoclave for 4 hours at 15 lb pressure.

The hydrolysate was treated and assayed as described in 'Experiment II.' All assays were conducted in duplicate.

Results and Discussion

The results obtained (Table 10) indicated that the biotin content of egg yolk was not affected by progression of the hatching season. Except for one instance (Hatch 2) the level of biotin in the yolks of eggs remained remarkably constant (361 to 378 mμg/g). In the second hatch the biotin content of the yolks was 278 mμg/g. The very low biotin content of one of the yolk samples was responsible for the low average value obtained.

The biotin content of livers of day-old poults tended to decline as the hatching season advanced. In Hatch 1 and 2, a biotin level of 4051 mμg/g of liver was noted. The level then declined steadily to a low of 3055 mμg/g in Hatch 5. It is difficult to reconcile the fact that biotin content of egg yolk remained relatively constant while that of day-old poults declined as the hatching season advanced. Nevertheless, the decline in biotin level in the livers of poults may be responsible for the increased incidence and severity of symptoms of biotin deficiency in poults hatched late in the hatching season.

Summary

Samples of yolks of turkey eggs and livers of day-old poults taken at 28-day intervals during the hatching season were assayed for their biotin content. The values obtained indicated the following:

(1) Biotin level in yolks of turkey eggs remained relatively constant throughout the hatching season.

(2) The level of biotin in the livers of day-old poults gradually decreased as the hatching season progressed.

(3) No relationship between the biotin level of yolks of turkey eggs and the biotin content of livers of day-old poults hatched from eggs produced at the same time was observed.

Table 10. Effect of progression of the hatching season on the biotin content of yolks of turkey eggs and livers of day-old poults.

Hatch No.		Biotin Content of Egg Yolks			Biotin Content of Livers of Day-old Poults		
		Sample 1 (mμg/g)	Sample 2 (mμg/g)	Average (mμg/g)	Sample 1 (mμg/g)	Sample 2 (mμg/g)	Average (mμg/g)
1	Jan. 21/69	358	364	361	3907	4195	4051
2	Feb. 18/69	235	321	278	4119	3983	4051
3	March 18/69	413	332	373	3544	4077	3811
4	April 15/69	388	350	369	3248	4204	3726
5	May 13/69	362	373	368	3240	2870	3055

IV. The Effects of Dietary Biotin Level and Ration Type on Storage of Biotin in Livers of 4-week-old Poults.

Status of the Problem

Although it is possible to measure the biotin content of a ration by microbiological assay, determination of the availability of biotin in the ration is difficult. It therefore would be useful if a method could be devised which would indicate, with some degree of accuracy, the amount of biotin in a ration that is available to the poult. If the assumption were made that available biotin in the ration has a direct effect on the amount of biotin stored in the liver, then the biotin content of liver should reflect the availability of biotin in the ration.

In order to determine whether this relationship exists, an experiment was initiated to study the effect of dietary biotin level and ration type on storage of biotin in the livers of 4-week-old poults.

Experimental

The composition of the rations fed are presented in Table 11. The rations differed in sources of protein supplementation and level of biotin used. In order to determine whether progression of the hatching season would affect the results obtained, the rations were fed to poults hatched early in the hatching season (February, 18th) and to poults hatched late in the hatching season (June, 10th). In the first trial, 4 comparable groups of 62 day-old poults and in the second trial 4 groups of 35 day-old poults were fed the experimental rations. The poults were reared until 4 weeks of age in radiant-heated floor pens using wheat straw as litter. The poults were individually weighed at 4 weeks of age and scored for symptoms of biotin deficiency.

At the end of the feeding trial, 4 poults were randomly selected

Table 11. Composition of turkey starter rations.

Ingredients	Ration Number			
	1 (%)	2 (%)	3 (%)	4 (%)
Ground Corn	10	10	10	10
Ground Wheat	46	46	38	38
Wheat Shorts	9.215	9.215	1.715	1.715
Stabilized animal fat	-	-	2	2
Dehydrated alfalfa meal	-	-	2	2
Meat Meal (55% Protein)	22	22	10	10
Herring Meal (72% Protein)	10	10	5	5
Soybean Meal (44% Protein)	-	-	28	28
Iodized Salt	0.25	0.25	0.25	0.25
Ground Limestone	1	1	1	1
Dicalcium Phosphate (18.5% Ca, 20.5% P)	-	-	0.5	0.5
Manganese Sulfate	0.025	0.025	0.025	0.025
Zinc Oxide	0.01	0.01	0.01	0.01
Micronutrient Mix[1]	1.5	1.5	1.5	1.5
Biotin[2]	-	+	-	+

[1]Micronutrient mix supplied the following levels per kg of ration:- Vitamin A, 5940 IU; Vitamin D_3, 1650 ICU; Vitamin E, 22 IU; menadione sodium bisulfate, 1.1 mg; riboflavin, 3.3 mg; calcium pantothenate, 11 mg; niacin, 22 mg; choline chloride, 187 mg; Vitamin B_{12}, 0.0066 mg; folic acid, 3.3 mg; penicillin, 4.4 mg; DL-methionine, 500 mg.

[2]Biotin additions were made at the level of 220 µg/kg.

fromeach treatment. The poults were killed and their livers were removed, pooled, homogenized and stored at -20 C until assayed for biotin content. In a similar fashion liver samples were taken from each treatment group of Trial 6, Experiment I. Samples of the rations fed in Experiment IV were also taken for determination of their biotin content. Duplicate samples of liver and feed were hydrolysed with 4 N H_2SO_4 for 4 and 2 hours respectively. The biotin content of the hydrolysates were then determined by microbiological assay using the same procedure as that outlined previously (Experiment II).

Results and Discussion

Data on average body weight, incidence of deficiency symptoms, biotin content of rations fed and liver storage of biotin in 4-week-old poults are summarized in Table 12.

The average body weights of poults was affected by the protein supplements included in the rations. When meat meal and fish meal were the only protein supplements used, rate of growth was considerably lower than when soybean meal was included (Groups 3 and 4 vs Groups 1 and 2). The addition of biotin had no effect on rate of growth in the first trial but in the second trial an increase in rate of growth was observed (Group 2 vs Group 1).

The biotin content of livers of poults was affected by protein supplements used and levels of biotin in the ration. In both trials the biotin content of liver was considerably lower in poults fed the rations containing meat meal and fish meal (Ration 1), than when soybean meal was included in the supplementary protein (Ration 3). This suggests the possibility that the availability of biotin in meat meal and fish meal may be low or that differences in the amino acid composition of the

Table 12. Effects of protein supplements and biotin additions on average body weight and biotin content of livers of 4-week-old poults.

Date of Hatch	Group No.	Treatment*	Observations at 4 Wks of Age					Biotin Content of Feed (mμg/g)	Biotin Content of Livers (mμg/g)
			Avg Body Wt (g)	Hock Disorder %	Dermatitis Beak and Eye %	Dermatitis Feet %	Broken Feathers %		
February 18/69	1	MF	424	54	31	56	59	210	1283
	2	MF + Biotin	449	2	0	16	7	330	1841
	3	MFS	598	0	0	16	0	261	2163
	4	MFS + Biotin	597	2	0	8	0	424	2852
June 10/69	1	MF	311	89	11	82	85	200	1112
	2	MF + Biotin	378	39	0	35	26	330	2256
	3	MFS	512	0	0	15	3	296	2340
	4	MFS + Biotin	537	0	0	30	0	541	2779

*M, F and S refer to meat meal, fish meal and soybean meal.

Table 13. Effect of biotin levels and sources of UGF on body weight and liver biotin content of poults.

Group No.	Biotin Added (µg/kg)	UGF Added	Calculated Biotin Levels in Feed		Avg Body Wt at 4 Wk of Age (g)	Biotin Content of Liver (mµg/g)
			Without UGF (µg/kg)	With UGF (µg/kg)		
1	0	-	52		292	834
2	0	+		96	426	503
3	100	-	152		530	2135
4	100	+		196	604	2095
5	200	-	252		482	2842
6	200	+		296	545	4635
7	300	-	352		510	2968
8	300	+		396	549	3253
9	400	-	452		521	3847
10	400	+		496	561	4192

ration may account for differences in biotin requirements. Addition of supplementary biotin resulted in marked increases in biotin content of liver in all instances (Ration 2 vs 1 and Ration 4 vs 3).

The biotin content of feeds and the storage of biotin in the liver were apparently not affected by the time during the hatching season at which the experiments were conducted. Nevertheless, average body weight attained at 4 weeks of age was considerably lower in poults hatched late in the hatching season than in those hatched early in the season.

Severe symptoms of a biotin deficiency were present in poults fed the basal ration containing meat meal and fish meal (Ration 1) in both early and late hatched poults. The addition of biotin to the basal (Ration 2) greatly reduced the symptoms in early hatched poults but did so to a lesser extent in later hatched poults. In poults fed rations containing soybean meal, with or without added biotin, (Rations 3 and 4) the incidence of deficiency symptoms was very low.

The biotin content of livers of poults fed purified and semi-purified rations containing graded levels of biotin (Trial 6, Experiment I) was determined and the values obtained as well as the calculated biotin levels of the ration and the average body weight of poults at 4 weeks of age are presented in Table 13. The data indicated that the biotin content of livers of poults tended to increase ($b = 0.85$) with increasing levels of biotin in the ration.

When deficiency symptoms (Table 8) were related to the calculated biotin content of the ration it appeared that the addition of 100 µg of biotin per kg, to a basal calculated to contain 96 µg of biotin per kg, was sufficiently high to prevent the appearance of the deficiency;

however, addition of 200 µg of biotin per kg of ration was required to prevent symptoms in the ration without sources of UGF.

Examination of the data of Tables 12 and 13 indicated that there was some relationship between the biotin content of liver and the deficiency symptoms. When the biotin content of the liver was 1283 mµg/g or less, severe symptoms of a biotin deficiency were noted: when the biotin content of liver was 1841 mµg/g or higher, symptoms of deficiency were greatly reduced or were absent.

Summary

Rations containing different protein supplements and levels of biotin were fed to poults at the beginning and end of the hatching season. In addition purified and semipurified rations with graded levels of biotin were also fed to other poults. Assays for biotin content were carried out on the rations fed and on the livers of poults at 4 weeks of age. The results indicated that:

(1) Inclusion of soybean meal in the ration resulted in a higher rate of growth with less symptoms of biotin deficiency than when meat meal and fish meal were used as the only protein supplements.

(2) Poults fed the basal ration containing soybean meal had higher levels of biotin in the liver than those fed the basal ration containing meat meal and fish meal.

(3) Addition of biotin to the rations resulted in increased liver biotin content.

(4) Time of the hatching season had no effect on the biotin storage in the liver.

(5) The biotin content of livers of poults tended to increase with increasing levels of biotin in the ration.

(6) When the level of biotin in the livers of 4-week-old poults was 1283 mμg/g or less, symptoms of a biotin deficiency were severe; when the biotin content was 1841 mμg/g or more, symptoms of deficiency were slight or absent.

GENERAL DISCUSSION

A purified ration that was satisfactory for normal growth and feathering of poults was developed. When biotin was omitted from the ration severe symptoms of a biotin deficiency occurred, which were prevented by the addition of biotin to the ration. It was evident, however, that the levels of biotin required to prevent deficiency symptoms varied from one feeding trial to the next. In different trials the optimum level of biotin supplementation ranged from 100 to 300 µg of biotin per kg of ration. It was therefore not possible, on the basis of these experiments, to estimate the biotin requirement of the poult accurately.

Average body weights of poults at 4 weeks of age appeared to be affected by the level of biotin supplementation used. Levels of biotin in excess of those required to prevent deficiency symptoms caused a reduction in average weight of the poults in some instances. This was similar to an observation made by Waibel *et al.* (1969) who noted that the addition of biotin in excess of requirements resulted in lower average body weight than was obtained on an unsupplemented ration.

The apparent variability in biotin requirements from one feeding trial to another, as well as the possible depressing effect of excess amounts of biotin in the ration, greatly complicates the problem of formulating feeds to meet the poult's dietary requirements for the vitamin. Until all the factors affecting the poult's dietary requirements for biotin are elucidated and their influence quantitated it will be difficult to estimate accurately the requirements of the poult for biotin.

Determination of the biotin content of feed ingredients used in poultry rations in Alberta indicated that the biotin content was generally similar to that reported elsewhere. Thus the suggestion that

the sporadic appearance of deficiency symptoms in Alberta might be related to lower levels of biotin in feed ingredients grown in the Province as compared to those used elsewhere does not appear to be valid.

Determination of the biotin content of a number of commercial turkey starter rations indicated that the levels of biotin ranged from 353 to 774 µg/kg of ration. These levels should be adequate to meet the poult's requirement for the vitamin under most circumstances.

The observation by Robblee and Clandinin (1953) that late-hatched poults were more susceptible to symptoms of biotin deficiency led to the investigation of the biotin content of yolks of turkey eggs and livers of day-old poults taken at 28-day intervals during the hatching season. The data indicated that biotin content of egg yolks was relatively constant throughout the hatching season but that of the livers gradually declined as the hatching season progressed. The decline in liver biotin content of day-old poults may account for the poorer performance of late hatched poults and their greater dependence on supplemental biotin. No explanation to account for the constant level of biotin in the yolks of eggs and the declining level of biotin in livers of day-old poults can be offered.

The biotin content of livers of 4-week-old poults tended to increase with increasing levels of biotin in the ration. In relating the biotin content of the liver to the status of biotin nutrition in poults, it was found that poults containing less than 1283 mµg of biotin per g of liver exhibited severe symptoms of a biotin deficiency, but when the biotin content was 1841 mµg/g or higher, symptoms of deficiency were greatly reduced or were absent. Appearance of deficiency symptoms in poults appeared to be related to the

type of protein supplement used and progression of the hatching season. Incidence of deficiency symptoms was higher when soybean meal was not included in the protein supplement used and tended to increase in poults late in the hatching season. Thus the problem of preventing the appearance of biotin deficiency syndrome in poults and of accurately estimating the biotin requirement of the poult is complicated by many factors.

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